

§ 113.331

9 CFR Ch. I (1–1–03 Edition)

vaccine shall be reconstituted in diluent according to the label recommendations, and held in an ice bath at 0°C to 4°C for 2 hours prior to use in the potency test.

(ii) For a serial or subserial to be eligible for release, each serotype contained in the vaccine shall have a virus titer per dose which is at least 3 times greater than the number of plaque forming units (pfu) used in the immunogenicity test prescribed in paragraph (c) of this section, but not less than 1000 pfu per dose.

(iii) When tested (without the pretest incubation of desiccated products) at any time within the expiration period, each serotype contained in the vaccine shall have a virus titer per dose which is at least 2 times the number of pfu used in the immunogenicity test, but not less than 750 pfu per dose.

[61 FR 33841, July 1, 1996]

§ 113.331 Bursal Disease Vaccine.

Bursal Disease Vaccine shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe, and immunogenic in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for preparing the production seed virus for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in § 113.300 and the requirements prescribed in this section.

(b) Each lot of Master Seed Virus shall be tested for pathogens by the chicken embryo inoculation test prescribed in § 113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in § 113.36 may be conducted and the virus judged accordingly. Each lot of Master Seed Virus used in the preparation of modified live virus vaccines shall also be nonpathogenic to chickens as determined by the following procedures:

(1) Each of twenty-five 1-day-old bursal disease susceptible chickens (vaccinates) shall be injected subcutaneously with 10 times the rec-

ommended dose of vaccine virus and observed for 21 days. Fifteen chickens of the same source and hatch shall be kept isolated as controls.

(i) Seventeen days postvaccination, each of five controls shall be administered at least $10^{2.0}$ EID₅₀ of a virulent bursal disease virus by eye-drop, isolated, and used as positive controls. The remaining controls shall be used as negative controls.

(ii) If the vaccinates do not remain free of clinical signs of bursal disease, the Master Seed Virus is unsatisfactory. If unfavorable reactions which are not attributable to the Master Seed Virus occur in more than two of the vaccinates, the test shall be declared inconclusive and may be repeated.

(iii) Twenty-one days postvaccination, the vaccinates and the controls shall be necropsied and examined for gross lesions of bursal disease. If more than two of the vaccinates have such lesions, the Master Seed Virus is unsatisfactory, except that, if any of the negative controls or less than four of the positive controls have such lesions, the test is inconclusive and may be repeated. For purposes of this test, gross lesions shall include obvious pathological processes and/or obvious reduction in size of the bursa from normal.

(2) Each of thirty-five 3- to 4-week-old bursal disease susceptible chickens (vaccinates) shall be vaccinated with approximately one minimum protective dose of vaccine virus as determined in paragraph (c) of this section. Each of 10 chickens of the same source and hatch shall be administered at least $10^{2.0}$ EID₅₀ of a virulent bursal disease virus by eye-drop, isolated, and used as positive controls. Also, each of 20 additional chickens of the same source and hatch shall be isolated and held as negative controls.

(i) Three or four days postvaccination, 10 of the vaccinates, the 10 positive controls, and 10 of the negative controls shall be necropsied and examined for gross lesions of bursal disease. If any of the vaccinates have such lesions, the Master Seed Virus is unsatisfactory, except that, if any of the negative controls or less than 8 of the positive controls have such lesions, the test is inconclusive

and may be repeated. For purposes of this test, gross lesions shall include peri-bursal edema and/or edema and/or macroscopic hemorrhage in the bursal tissue.

(ii) Fourteen days post-vaccination, the remaining vaccinates and negative controls shall be necropsied and examined for obvious bursal atrophy. If any of the vaccinates have such atrophy, the Master Seed Virus is unsatisfactory, except that, if any of the negative controls have such atrophy, the test is inconclusive and may be repeated.

(c) Each lot of Master Seed Virus shall be tested for immunogenicity and the selected virus dose to be used shall be established as follows:

(1) Bursal Disease susceptible chickens, all of the same age (3 weeks or younger) and from the same source, shall be used. Twenty or more chickens shall be used as vaccinates for each method of administration recommended on the label. Ten additional chickens of the same age and from the same source shall be held as unvaccinated controls.

(2) A geometric mean titer of the vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. Each vaccinee shall receive a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on an aliquot of the vaccine virus to confirm the amount of virus administered to each chicken used in the test. At least three appropriate (not to exceed ten-fold) dilutions shall be used to conduct the titrations by a method acceptable to Animal and Plant Health Inspection Service.

(3) When the test chickens are 28 to 35 days of age but not less than 14 days postvaccination, each vaccinee and each control shall be challenged by eye-drop with a virulent bursal disease virus provided or approved by Animal and Plant Health Inspection Service.

(i) Three to five days postchallenge, all vaccinates and controls shall be necropsied and examined for gross lesions of bursal disease as described in paragraph (b)(2)(i) of this section.

(ii) If at least 19 of 20, or 27 of 30, or 36 of 40 vaccinates in each group are not free from such lesions, the Master

Seed Virus is unsatisfactory, except that, if less than 90 percent of the controls have such lesions, the test is inconclusive and may be repeated.

(4) The Master Seed Virus shall be retested for immunogenicity in 3 years from the original testing unless use of the lot previously tested is discontinued. Only one method of administration recommended on the label need be used in the retest. The vaccinates and the controls shall meet the criteria prescribed in paragraph (c)(3) of this section.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) After a lot of Master Seed Virus has been established as prescribed in paragraphs (a), (b), and (c) of this section, each serial and subserial shall meet the applicable requirements in § 113.300 and the requirements prescribed in this paragraph.

(1) *Tests for pathogens.* Final container samples from each serial shall be tested for pathogens by the chicken embryo inoculation test prescribed in § 113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in § 113.36 may be conducted and the serial judged accordingly.

(2) *Safety tests.* (i) Final container samples of completed product from each serial shall be tested to determine whether the vaccine is safe as follows:

(A) For vaccines intended for parenteral administration, each of twenty-five 1-day-old bursal disease susceptible chickens shall be vaccinated with the equivalent of 10 doses by subcutaneous injection.

(B) For vaccines intended for drinking water administration, each of twenty-five 4- to 5-week-old bursal disease susceptible chickens shall be vaccinated orally with the equivalent of 10 doses.

(C) Ten chickens of the same source and hatch shall be maintained in isolation as negative controls. The vaccinates and controls shall be observed each day for 21 days.

(ii) If unfavorable reactions which are attributable to the biological product occur during the observation period, the serial is unsatisfactory. If unfavorable reactions occur in more than one of the controls or if unfavorable reactions which are not attributable to the biological product occur in more than two of the vaccinates, the test shall be declared inconclusive and repeated, except that, if the test is not repeated, the serial shall be unsatisfactory.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ times greater than that used in such immunogenicity test, but not less than $10^{2.0}$ titration units (PFU or ID₅₀'s) per dose.

[44 FR 60263, Oct. 19, 1979, as amended at 44 FR 67087, Nov. 23, 1979; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 64 FR 43045, Aug. 9, 1999]

§ 113.332 Tenosynovitis Vaccine.

Tenosynovitis Vaccine shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs.

Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.300, except (a)(3)(ii) and (c), and the special requirements in this section.

(b) Each lot of Master Seed shall be tested for:

(1) Pathogens by the chicken inoculation test prescribed in § 113.36.

(2) Lymphoid leukosis virus contamination as follows:

(i) Each of at least 10 3-week-old or older lymphoid leukosis free chickens from the same source and hatch shall be injected intra-muscularly with an amount of Master Seed equal to 100 label doses of vaccine. At least 15 chickens of the same source and hatch shall be used as controls; 5 or more shall be unvaccinated and serve as negative controls; 5 or more shall be injected with subgroup A lymphoid leukosis virus; and 5 or more with subgroup B lymphoid leukosis virus. Each group of control chickens shall be held isolated from each other and from the vaccinates.

(ii) Twenty-one to 28 days postinoculation, blood samples shall be taken from each chicken and the serum separated using a technique conducive to virus preservation. These serums shall be used as inocula in the complement fixation for avian lymphoid leukosis (COFAL) test prescribed in § 113.31.

(iii) Serums from the vaccinates shall be tested separately, but serums within each control group may be pooled. A valid test shall have positive COFAL reactions from each virus inoculated group and negative reactions from the uninoculated controls. If any of the chickens injected with the Master Seed have positive COFAL test reactions in a valid test, the Master Seed is unsatisfactory.

(3) Identity using the following agar gel immunodiffusion test. The undiluted Master Seed may be used as test antigen or the Master Seed may be inoculated onto the chorioallantoic membrane (CAM) of fully susceptible chicken embryos and the infected CAMs ground and used as antigen. A known tenosynovitis antiserum and a known tenosynovitis antigen shall be used in the test. A precipitin line shall form between the test antigen and the known antiserum in the center well which shows identity with the line formed between the antiserum and the known antigen, or the Master Seed is unsatisfactory.

(4) Safety using the following chicken test:

(i) For vaccines intended for use in chickens less than 14 days of age, Master Seed equal to 10 label doses shall be administered subcutaneously to each of